

SEQUENCE LISTING

<110> SES EUROPE N.V./S.A.

<120> METHOD OF GENETIC MODIFICATION OF A WILD TYPE VIRAL
SEQUENCE

<130> P.SES.02/WO

<140>

<141>

<160> 6

<170> PatentIn Ver. 2.1

<210> 1

<211> 399

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)..(399)

<220>

<223> Description of Artificial Sequence: genetically
modified TGB-3 viral sequence

<400> 1

atg gtg ctt gtg gtt gca gta gct tta tct aat att gta ttg tac ata 48
Met Val Leu Val Val Ala Val Ala Leu Ser Asn Ile Val Leu Tyr Ile
1 5 10 15

gtt gcc ggt tgt gtt gtt gtc agt atg ttg tac tca ccg ttt ttc agc 96
Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
20 25 30

aac gat gtt aaa gcg tcc agc tat gcg gga gca att ttt aag ggg agc 144
Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
35 40 45

ggc tgt atc atg gac agg aat tgg ttt gct caa ttt ggg agt tgc gat 192
Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
50 55 60

att cca aag cat gta gcc gag tcc atc act aag gtt gcc acc aaa gag 240
Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu

65	70	75	80	
cac gat gtt gac ata atg gta aaa agg ggt gaa gtg acc gtt cgt gtt				288
His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val				
	85	90	95	
gtg act ctc acc gaa act att ttt ata ata tta tct aga ttg ttt ggt				336
Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly				
	100	105	110	
ttg gcg gtg ttt ttg ttc atg ata tgt tta atg tct ata gtt tgg ttt				384
Leu Ala Val Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe				
	115	120	125	
tggttatcatagataa				399
Trp Tyr His Arg				
130				

<210> 2

<211> 132

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: genetically
modified TGB-3 viral sequence

<400> 2

Met Val Leu Val Val Ala Val Ala Leu Ser Asn Ile Val Leu Tyr Ile				
1	5	10	15	
Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser				
	20	25	30	
Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser				
	35	40	45	
Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp				
	50	55	60	
Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu				
	65	70	75	80
His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val				
	85	90	95	
Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly				
	100	105	110	

Leu Ala Val Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
 115 120 125

Trp Tyr His Arg
 130

<210> 3

<211> 399

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)..(399)

<220>

<223> Description of Artificial Sequence: genetically
 modified TGB-3 viral sequence

<400> 3

atg gtg ctt gtg gtt aaa gta gat tta tct aat att gta ttg tac ata 48
 Met Val Leu Val Val Lys Val Asp Leu Ser Asn Ile Val Leu Tyr Ile
 1 5 10 15

gtt gcc ggt tgt gtt gtt gtc agt atg ttg tac tca ccg ttt ttc agc 96
 Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
 20 25 30

aac gat gtt aaa gcg tcc agc tat gcg gga gca att ttt aag ggg agc 144
 Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
 35 40 45

ggc tgt atc atg gcc gcg aat tcc ttt gct caa ttt ggg agt tgc gat 192
 Gly Cys Ile Met Ala Ala Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
 50 55 60

att cca aag cat gta gcc gag tcc atc act aag gtt gcc acc aaa gag 240
 Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
 65 70 75 80

cac gat gtt gac ata atg gta aaa agg ggt gaa gtg acc gtt cgt gtt 288
 His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
 85 90 95

gtg act ctc acc gaa act att ttt ata ata tta tct aga ttg ttt ggt 336
 Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly

100

105

110

ttg gcg gtg ttt ttg ttc atg ata tgt tta atg tct ata gtt tgg ttt 384
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 115 120 125

tgg tat cat aga taa 399
 Trp Tyr His Arg
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<210> 4

<211> 132

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: genetically
 modified TGB-3 viral sequence

<400> 4

Met Val Leu Val Val Lys Val Asp Leu Ser Asn Ile Val Leu Tyr Ile
 1 5 10 15

Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
 20 25 30

Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
 35 40 45

Gly Cys Ile Met Ala Ala Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
 50 55 60

Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
 65 70 75 80

His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
 85 90 95

Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
 100 105 110

Leu Ala Val Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
 115 120 125

Trp Tyr His Arg
 130

<210> 5
 <211> 399
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)..(399)

<220>
 <223> Description of Artificial Sequence: genetically
 modified TGB-3 viral sequence

<400> 5
 atg gtg ctt gtg gtt aaa gta gat tta tct aat att gta ttg tac ata 48
 Met Val Leu Val Val Lys Val Asp Leu Ser Asn Ile Val Leu Tyr Ile
 1 5 10 15
 gtt gcc ggt tgt gtt gtt gtc agt atg ttg tac tca cgg ttt ttc agc 96
 Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
 20 25 30
 aac gat gtt aaa gcg tcc agc tat gcg gga gca att ttt aag ggg agc 144
 Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
 35 40 45
 ggc tgt atc atg gac agg aat tcg ttt gct caa ttt ggg agt tgc gat 192
 Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
 50 55 60
 att cca aag cat gta gcc gag tcc atc act aag gtt gcc acc aaa gag 240
 Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
 65 70 75 80
 cac gat gtt gac ata atg gta aaa agg ggt gaa gtg acc gtt cgt gtt 288
 His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
 85 90 95
 gtg act ctc acc gaa act att ttt ata ata tta tct aga ttg ttt ggt 336
 Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
 100 105 110
 ttg gat gat ttt ttg ttc atg ata tgt tta atg tct ata gtt tgg ttt 384
 Leu Asp Asp Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
 115 120 125
 tgg tat cat aga taa 399
 Trp Tyr His Arg

130

<210> 6

<211> 132

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: genetically
modified TGB-3 viral sequence

<400> 6

Met Val Leu Val Val Lys Val Asp Leu Ser Asn Ile Val Leu Tyr Ile
1 5 10 15

Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
20 25 30

Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
35 40 45

Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
50 55 60

Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
65 70 75 80

His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
85 90 95

Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
100 105 110

Leu Asp Asp Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
115 120 125

Trp Tyr His Arg
130

CLAIMS

1. Method of genetic modification of a TGB-3 wild type viral sequence for reducing or suppressing the possible deleterious effects of the agronomic properties of a transformed plant or plant cell by said TGB-3 viral sequence, comprising the following successive steps :
- submitting said sequence to point mutation(s) which allow the substitution of at least one amino-acid into a different amino-acid,
 - 10 - selecting genetically modified TGB-3 wild type viral sequences having said point mutation(s) and which are not able to promote cell-to-cell movement of a mutant virus having a dysfunctional TGB-3 wild type viral sequence, when expressed in trans from a replicon,
 - 15 - further selecting among said genetically modified TGB-3 viral sequences, the specifically genetically modified sequence which inhibits infection with a co-inoculated wild type virus when the mutant form was expressed from a replicon, and
 - 20 - recovering said specifically genetically modified TGB-3 viral sequence.

2. Method according to claim 1, wherein the TGB-3 wild type viral sequence is the BNYVV P15 sequence.

3. Genetically modified TGB-3 viral sequence
25 obtained by the method according to claim 1 or 2.

4. Genetically modified TGB-3 viral sequence according to claim 3, being selected from the group consisting of the following sequences :

SEQ ID NO 1 :

30 ATGGTGCTTGTGGTTGGAGCTAGCTTTATCTAATATTGTTATTTACATAGTTGCCCGTTGT 60
M V L V V A V A L S N I V L Y I V A G C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V V V S M L Y S P F F S N D V K A S S Y

GCGGGAGCAATTTTAAAGGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180
5 A G A I F K G S G C I M D R N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G S C D I P K H V A E S I T K V A T K E

10 CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTGTTCATGATA 360
E T I F I I L S R L F G L A V F L F M I

15 TGTTTAATGTCTATAGTTTGGTTTGGTATCATAGATAA 399
C L M S I V W F W Y H R *

SEQ ID NO 2 :

20 ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTGT 60
M V L V V K V D L S N I V L Y I V A G C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V V V S M L Y S P F F S N D V K A S S Y

25 GCGGGAGCAATTTTAAAGGGGAGCGGCTGTATCATGGCCCGAATTCGTTTGCTCAATTT 180
A G A I F K G S G C I M A A N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
30 G S C D I P K H V A E S I T K V A T K E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTTGTTCATGATA 360
E T I F I I L S R L F G L A V F L F M I

TGTTTAATGTCTATAGTTTGGTTTGGTATCATAGATAA 399
5 C L M S I V W F W Y H R *

SEQ ID NO 3 :

ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60
M V L V V K V D L S N I V L Y I V A G C

10 GTTGTGTCAGTATGTTGTAAGTCAACGATGTTAAAGCGTCCAGCTAT 120
V V V S M L Y S P F F S N D V K A S S Y

GCGGGAGCAATTTTAAAGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180
15 A G A I F K G S G C I M D R N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G S C D I P K H V A E S I T K V A T K E

20 CACGATGTTGACATAATGCTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTATAATATTATCTAGATTGTTTGGTTTGGATGATTTTTTGTTCATGATA 360
E T I F I I L S R L F G L D D F L F M I

25 TGTTTAATGTCTATAGTTTGGTTTGGTATCATAGATAA 399
C L M S I V W F W Y H R *

5. Vector comprising the genetically modified
30 TGB-3 viral sequence according to the claim 3 or 4,
possibly linked to one or more regulatory sequence(s)
capable of being active into a plant or a plant cell.

6. Method for inducing resistance into a plant or a plant cell to a virus comprising a TGB-3 sequence, comprising the following steps :

- preparing a nucleic acid construct comprising a
5 genetically modified TGB-3 viral sequence according to claim 4 or 5, being operably linked to one or more regulatory sequence(s) capable of being active into a plant or a plant cell,
- transforming a plant cell with said nucleic acid
10 construct, and possibly
- regenerating a transgenic plant from the transformed plant cell.

7. Method according to claim 6, characterised in that the virus is selected from the group consisting of
15 the apple stem pitting virus, the blueberry scorch virus, the potato virus M, the white clover mosaic virus, the *Cymbidium* mosaic virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus, the beet soil-borne virus or the BNYVV virus.

20 8. Method according to claim 6 or 7, characterised in that the plant cell is a stomatal cell.

9. Method according to any one of the claims 6 to 8, characterised in that the plant is selected from the group consisting of apple, blueberry, potato, clover,
25 orchid, barley, peanut or sugar beet.

10. Method according to any one of the claims 6 to 9, characterised in that the regulatory sequence comprises a promoter sequence or a terminator sequence active in a plant.

30 11. Method according to claim 10, characterised in that the promoter sequence is a constitutive or a foreigner promoter sequence.

12. Method according to claim 10, characterised in that the promoter sequence is selected from the group consisting of 35S Cauliflower Mosaic Virus promoter, and/or the polyubiquitin *Arabidopsis thaliana* promoter.

13. Method according to any one of the claims 10 to 12, characterised in that the promoter sequence is a promoter which is capable of being active mainly into the root tissue of plants such as the par promoter of the haemoglobin gene from *Perosponia andersonii*.

14. Transgenic plant or transgenic plant cell resistant to a virus and comprising a nucleic acid construct having a genetically modified TGB-3 viral sequence according to claim 4 or 5, being operably linked to one or more regulatory sequence(s) active into a plant or a plant cell.

15. Transgenic plant or transgenic plant cell according to claim 14, characterised in that the virus is selected from the group consisting of the apple stem pitting virus, the blueberry scorch virus, the potato virus M, the white clover mosaic virus, the *Cymbidium* mosaic virus, the potato virus X, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus, the beet soil-borne virus and the BNYVV virus.

16. Transgenic plant or transgenic plant cell according to claim 14 or 15, being a plant or a plant cell selected from the group consisting of apple, blueberry, potato, clover, orchid, barley, peanut or sugar beet plant or plant cell.

17. Transgenic plant or transgenic plant cell according to any one of the claims 14 to 16, characterised in that the regulatory sequence comprises a promoter

sequence and a terminator sequence capable of being active into a plant.

18. Transgenic plant or transgenic plant cell according to any one of the claims 14 to 17, characterised
5 in that the regulatory sequence(s) comprise a promoter sequence which is a constitutive or a foreigner vegetal promoter sequence.

19. Transgenic plant or transgenic plant cell according to claim 18, characterised in that promoter
10 sequence is selected from the group consisting of 35S Cauliflower Mosaic Virus promoter, and/or the polyubiquitin Arabidopsis thaliana promoter.

20. Transgenic plant or transgenic plant cell according to claim 18 or 19, characterised in that the
15 promoter sequence is a promoter which is mainly active in root tissues such as the par promoter of the haemoglobin gene from *Perosponia andersonii*.

21. Transgenic plant tissue selected from the group consisting of fruit, stem, root, tuber, seed of a
20 plant according to any one of the claims 14 to 20.

22. Reproducible structure obtained from a transgenic plant according to any one of the claims 14 to
21.